

Please type a plus sign (+) inside this box → PTO/SB/05 (2/98)
Approved for use through 09/30/2000. OMB 0651-0032

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))		Attorney Docket No. PO3496US1
		First Inventor or Application Identifier Ostlund, Richard E. J.
		Title SITOSTANOL FORMULATION WITH EMULSIFIER...
		Express Mail Label No. EL193494343US

APPLICATION ELEMENTS See MPEP chapter 600 concerning utility patent application contents.		ADDRESS TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231									
<p>1. <input checked="" type="checkbox"/> * Fee Transmittal Form (e.g., PTO/SB/17) (Submit an original and a duplicate for fee processing)</p> <p>2. <input checked="" type="checkbox"/> Specification [Total Pages 21] (preferred arrangement set forth below)</p> <ul style="list-style-type: none"> - Descriptive title of the Invention - Cross References to Related Applications - Statement Regarding Fed sponsored R & D - Reference to Microfiche Appendix - Background of the Invention - Brief Summary of the Invention - Brief Description of the Drawings (if filed) - Detailed Description - Claim(s) - Abstract of the Disclosure <p>3. <input type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets]</p> <p>4. Oath or Declaration [Total Pages]</p> <p>a. <input checked="" type="checkbox"/> Newly executed (original or copy)</p> <p>b. <input type="checkbox"/> Copy from a prior application (37 C.F.R. § 1.63(d)) (for continuation/divisional with Box 17 completed) <i>[Note Box 5 below]</i></p> <p>i. <input type="checkbox"/> DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).</p> <p>5. <input type="checkbox"/> Incorporation By Reference (useable if Box 4b is checked) The entire disclosure of the prior application, from which a copy or the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.</p>		<p>6. <input type="checkbox"/> Microfiche Computer Program (Appendix)</p> <p>7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)</p> <ul style="list-style-type: none"> a. <input type="checkbox"/> Computer Readable Copy b. <input type="checkbox"/> Paper Copy (identical to computer copy) c. <input type="checkbox"/> Statement verifying identity of above copies 									
ACCOMPANYING APPLICATION PARTS											
<p>8. <input type="checkbox"/> Assignment Papers (cover sheet & document(s))</p> <p>9. <input type="checkbox"/> 37 C.F.R. § 3.73(b) Statement (when there is an assignee) <input type="checkbox"/> Power of Attorney</p> <p>10. <input type="checkbox"/> English Translation Document (if applicable)</p> <p>11. <input checked="" type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations</p> <p>12. <input type="checkbox"/> Preliminary Amendment</p> <p>13. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) (Should be specifically itemized) (2)</p> <p>14. <input checked="" type="checkbox"/> Small Entity Statement(s) <input type="checkbox"/> Statement filed in prior application, (PTO/SB/09-12) Status still proper and desired</p> <p>15. <input type="checkbox"/> Certified Copy of Priority Document(s) (if foreign priority is claimed)</p> <p>16. <input checked="" type="checkbox"/> Other: Cert. of Mailing by Post Office Express Mail (2).....</p>											
<p>NOTE FOR ITEMS 1 & 14: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).</p>											
<p>17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment:</p> <p><input type="checkbox"/> Continuation <input type="checkbox"/> Divisional <input checked="" type="checkbox"/> Continuation-in-part (CIP) of prior application No. 09 / 084,561</p> <p>Prior application information: Examiner Jordan, K. Group / Art Unit: 1614</p>											
18. CORRESPONDENCE ADDRESS											
<p><input type="checkbox"/> Customer Number or Bar Code Label (insert Customer No. or Attach bar code label here)</p>		<p>or <input checked="" type="checkbox"/> Correspondence address below</p>									
Name	Edmund J. Sease										
Address	801 Grand Avenue Suite 3200										
City	Des Moines	State	Iowa								
Country	USA	Telephone	515-288-3667								
Telephone			Fax 515-288-1338								
<table border="1"> <tr> <td>Name (Print/Type)</td> <td>Edmund J. Sease</td> <td>Registration No. (Attorney/Agent)</td> <td>24,741</td> </tr> <tr> <td>Signature</td> <td colspan="2"></td> <td>Date Feb. 15, 1999</td> </tr> </table>		Name (Print/Type)	Edmund J. Sease	Registration No. (Attorney/Agent)	24,741	Signature			Date Feb. 15, 1999		
Name (Print/Type)	Edmund J. Sease	Registration No. (Attorney/Agent)	24,741								
Signature			Date Feb. 15, 1999								

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.

09/25/07/99
02/15/99

Applicant or Patentee: Richard E. Ostlund, Jr.
Serial No. or Patent No: _____
Filed or Issued: _____
For: SITOSTANOL FORMULATION WITH EMULSIFIER TO REDUCE CHOLESTEROL ABSORPTION
AND METHOD FOR PREPARING AND USE OF SAME

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
37 CFR 1.9(f) AND 1.27(d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION Washington University
ADDRESS OF ORGANIZATION Campus Box 8127, 660 South Euclid Avenue, St. Louis, MO 63110

TYPE OF ORGANIZATION

UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
 TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3))

NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE
UNITED STATES OF AMERICA

(NAME OF STATE _____)

(CITATION OF STATUTE _____)

WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE
(26 USC 501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA

WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE
OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)

(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled SITOSTANOL FORMULATION WITH EMULSIFIER...

by inventor(s) Richard E. Ostlund, Jr. as described in

the specification filed herewith.

application serial no. _____, filed _____.

patent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

FULL NAME _____
ADDRESS _____
 INDIVIDUAL SMALL BUSINESS CONCERN NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
 INDIVIDUAL SMALL BUSINESS CONCERN NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of payment, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)). I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Andrew Neighbour
TITLE IN ORGANIZATION Associate Vice Chancellor for Technology Management
ADDRESS OF PERSON SIGNING Campus Box 8127, 660 South Euclid Ave., St. Louis, MO 63110
SIGNATURE Andrew Neighbour DATE 2/15/99

INVENTOR: RICHARD E. OSTLUND, JR.

TITLE: SITOSTANOL FORMULATION WITH EMULSIFIER TO REDUCE CHOLESTEROL ABSORPTION AND METHOD FOR PREPARING AND USE OF SAME

CROSS-REFERENCE TO A RELATED APPLICATION

This application is a continuation-in-part of Serial No. 09/084,561 filed May 26, 1998, now U.S. Patent _____, issued _____.

FIELD OF THE INVENTION

This invention relates to a composition and method for reducing cholesterol absorption and serum cholesterol in humans. It represents an improvement over my parent application.

BACKGROUND OF THE INVENTION

Phytosterols are plant sterols structurally similar to cholesterol that have been known for many years to reduce cholesterol absorption and serum cholesterol levels while not being absorbed themselves. Lowering of circulating cholesterol and low density lipoprotein cholesterol is an important part of a strategy to prevent and treat cardiovascular disease and especially coronary heart disease. Cholesterol absorption is a critical component of whole body cholesterol metabolism. Cholesterol derived from the diet and also from endogenous biliary secretion enters the intestine, and approximately 50% of the mixed intestinal load is absorbed, Bosner, M.S., Ostlund, R.E., Jr., Osofisan, O., Grosklos, J., Fritschle, C., Lange, L.G. 1993. The failure to absorb cholesterol quantitatively is therefore a key mechanism for the elimination of cholesterol from the body.

Drugs commonly used to treat high cholesterol levels have little or no effect on cholesterol absorption. For example, the potent new hydroxymethylglutaryl coenzyme A reductase inhibitors have a primary action to reduce

00250769-0021599

cholesterol synthesis rather than increase cholesterol elimination. Bile acid sequestrants such as the ion-exchange resin cholestyramine act within the intestine but do not bind cholesterol and may actually increase cholesterol absorption when given chronically. McNamara, D.J., N.O. Davidson, P. Samuel, and E.H. Ahrens, Jr. 1980, Cholesterol absorption in man: effect of administration of clofibrate and/or cholestyramine. *J. Lipid Res.* 21:1058-1064. Although orally-administered neomycin reduces cholesterol absorption effectively, it is toxic and has the disadvantage of requiring chronic administration of a potent antibiotic, Samuel, P. 1979. Treatment of hypercholesterolemia with neomycin--A time for reappraisal. *N. Engl. J. Med.* 301:595-597. The drug Cytellin®, an aqueous suspension of mixed phytosterols, was produced by Eli Lilly Co. for treatment of elevated cholesterol, but it has not been sold since 1985. As seen, it is apparent that new inhibitors of cholesterol absorption would complement currently-available treatment for high serum cholesterol.

Since phytosterols are natural products which are non-toxic and inexpensive byproducts of food processing, they may be important in the treatment of individuals with mildly-increased serum cholesterol, or for the general population in food products or dietary supplements. The use of phytosterols could reduce the need for systemically-absorbed drugs.

Despite their potential attractiveness, the usefulness of phytosterols has been limited by small and erratic effectiveness and a large dosage requirement. For example, doses of 5-18 g sitosterol/day reduced serum cholesterol by 16-20%. Farquhar, J.W. and M. Sokolow, 1958. A dose-response study showed that 3-9 g/day of powdered sitosterol was needed to decrease serum cholesterol levels by 12%. Lees, A.M., H.Y.I. Mok, R.S. Lees, M.A. McCluskey, and S.M. Grundy. 1977. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance, *Atherosclerosis* 28:325-338. To reduce the amount

needed, recent experiments have used sitostanol instead of sitosterol because it appears to be more potent than other phytosterols and is non-absorbable, Sugano,J.,H.Morioka, and I.Ikeda.(1977) A comparison of hypocholesterolemic activity of β -sitosterol and β -sitostanol in rats. J.Nutr.107:2011-2019. In subjects with severe hypercholesterolemia sitostanol at 1.5 g/day reduced serum cholesterol by 15%, Heinemann,T.,O.Leiss, and K.von Bergmann (1986) Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 61:219-223. However, sitostanol at 3 g/day had no effect in subjects with moderate hypercholesterolemia. Denke,M.A. (1995), Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Am.J.Clin.Nutr. 61:392-396.

Several investigators have proposed ways to increase the solubility or bioavailability of phytosterols in order to make them more useful. Based on studies in rats and the finding that phytosterol esters are much more soluble in oil than the free sterols, it has been proposed to use phytosterol esters in oil to lower cholesterol absorption, Mattson,F.H., R.A. Volpenhein, and B.A.Erickson (1977), Effect of plant sterol esters on the absorption of dietary cholesterol. J.Nutr. 107:1139-1146. U.S. Patent 5,502,045 describes the use of sitostanol ester in oil for the treatment of hypercholesterolemia in humans. It was found that 2.8 g sitostanol/day given as sitostanol ester in margarine reduced LDL cholesterol by 16%. Miettinen,T.A., P.Puska, H.Gylling, H.Vanhainen, and E.Vartiainen (1995), Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N.EnglandJ.Med. 333:1308-1312. However, the use of sitostanol ester dissolved in dietary fat has the disadvantage of requiring the administration of 23-50 g/day of dietary fat and of being 21% less effective at reducing cholesterol absorption in humans compared to the unesterified sterol. Mattson,F.H.,S.M.Grundy, and J.R.Crouse, (1982),

Optimizing the effect of plant sterols on cholesterol absorption in man. Am. J. Clin. Nutr. 35:697-700.

Additional workers have investigated ways to improve the usefulness of unesterified phytosterols. In International patent Publication WO 95/00158 a complex of sitosterol and the unabsorbable dietary fiber pectin reduced serum cholesterol by 16.4% when given to hypercholesterolemic humans in a dose of 2.1 g/day. However, no measurements of an effect on cholesterol absorption were made, and the complex was only about 50% soluble even at strongly alkaline pH, suggesting that the bioavailability of the sitosterol component was limited.

U.S. Patent 5,244,887 describes the use of stanols including sitostanol in food additives to reduce cholesterol absorption. In U.S. Patent 5,244,887, for preparation of the additives, sitostanol is dissolved with an edible solubilizing agent such as triglyceride, an antioxidant such as tocopherol, and a dispersant such as lecithin, polysorbate 80, or sodium lauryl sulfate. However, no data were given to guide one in the selection of the most effective components and their amounts or specific methods of preparation. Effectiveness in reducing cholesterol absorption was also not determined. The preferred embodiment consisted of 25% by weight stanols in vegetable oil, but the solubility of sterols in oil is only 2%.

U.S. Patent 5,118,671 describes the production of sitosterol-lecithin complexes for pharmaceutical use but does not consider oral use for cholesterol lowering.

Cholesterol is absorbed from an intestinal micellar phase containing bile salts and phospholipids which is in equilibrium with an oil phase inside the intestine. Delivery of phytosterol as a solid powder or aqueous suspension is not preferred because of the limited rate and extent of solubility in intestinal liquid phases. Esterification of the phytosterol with delivery through the oil phase of foods is an alternative route but has the disadvantage of use of edible oils as the carrier.

Accordingly, it is an object of the present invention to provide a delivery system for plant sterols, particularly sitostanol, which avoids an oil phase and which provides bioavailable sitostanol at a level which reduces cholesterol absorption as much as 37%, while at the same time using an excellent taste emulsifier in as low amounts as possible.

Another objective of the present invention is to provide a water soluble composition which provides the sitostanol, not dissolved in fat, but rather combined with a preferred emulsifier (Sodium Stearoyl 2-lactylate)(SSL) in an aqueous vesicular complex which can enter directly into the intestinal micellar phase and is therefore highly bioavailable.

Another objective of the present invention is to provide a composition of preferred enhanced solubility that contains a plant sterol, preferably sitostanol mixed with an emulsifier even better than phospholipids, namely SSL, which has water solubility in excess of 90%.

Another objective of the present invention is to provide a method for reducing cholesterol absorption from food products containing cholesterol by mixing finely divided water soluble powder of an aqueous homogeneous micellar mix of sitostanol and SSL with a food product which is to be ingested.

A yet further objective of the present invention is to provide a method of manufacturing a dry, finely divided water soluble powder which contains a plant sterol, preferably sitostanol, and lecithin, which is highly water soluble, so that when in contact with an aqueous system it will provide an aqueous vesicular complex which can enter directly into the intestinal micellar phase to inhibit cholesterol absorption.

The method and manner of achieving each of the above objectives, as well as others, will become apparent from the detailed description of the invention which follows hereinafter.

SUMMARY OF THE INVENTION

A composition for inhibiting cholesterol absorption from the intestine is described. The composition comprises phytosterols, preferably sitostanol, dispersed in an aqueous base emulsifier, preferably SSL. The mole ratio of sterol to emulsifier should be 1:0.1 to 1:10, preferably 1:0.9 to 1:0.5.

The phytosterol-emulsifier complex is prepared by high shear mixing, for example by vortexing, mixing, sonicating or passing through a small orifice of a phytosterol:emulsifier mixture in water. The dispersed material is then either used as is or dried, for example, by lyophilization or spray-drying. The complex can be used in liquid form prior to any drying, or it can be dried as indicated, and then on contact with liquid it again forms an aqueous vesicular complex which can enter directly into the intestinal micellar phase. No fat is used as a carrier, and surprisingly the system, even when dried, does not change its physical structure from the micelles that contain vesicles, the majority of which contain some plant sterol and some lecithin.

DETAILED DESCRIPTION OF THE INVENTION

As previously mentioned, the current invention differs from prior art uses of plant sterols and sitostanol in many significant ways.

First, the dose needed to reduce cholesterol absorption is lower than previously reported, namely 25-300 mg of sitostanol. Second, the preferred formulation does not contain triglycerides or oils. The phytosterol is not dissolved in fat, but rather is combined with phospholipid to form an aqueous vesicular complex which can enter directly into the intestinal micelle phase. Third, the mix can be prepared in solid form by drying an aqueous sitostanol/emulsifier vesicular formulation with retention of solubility in artificial bile. Fourth, the mix is effective when consumed separately from cholesterol-containing foods.

Fifth, the mix can be added to non-cholesterol-containing and fat-free foods and beverages. Sixth, the mix is prepared in a manner to prevent self-association of sitostanol as occurs when it is dried from organic solvents containing sitostanol and solubilizing agents. The mix herein referenced has the advantage of a high degree of bioavailability as assayed with artificial bile *in vitro*. This is significant and something that cannot be achieved with fat carrier systems.

The composition is useful for reducing cholesterol absorption in humans at doses between 10 and 1000 mg, and a preferred dose is 100-300 mg. The dose is less than required by current protocols. The composition may be used in capsule or tablet form as a drug or dietary supplement.

Alternatively, it may be used in foods as a food additive or substance generally recognized as safe for human consumption.

In preparation of the composition useful for reducing cholesterol in highly bioavailable form, the first step is to provide an aqueous homogeneous micellar mix of the plant sterol with the preferred emulsifier of choice.

The preferred method is to use sitostanol because only small amounts are absorbed in the small intestine, but on the other hand, this plant sterol shows high inhibition of cholesterol absorption. Similar compounds are also suitable, including sitosterol, campesterol, campestanol, stigmasterol. Moreover, lignans, such as sesamin, and saponins are also useful for this purpose, but sitostanol is preferred.

The preferred phospholipid of my parent case was lecithin, and the most preferred phospholipid system useful to enhance the bioavailability was a mix of lecithin and lysolecithin. Where the mix was used, it was preferred that the mole ratio of lecithin to lysolecithin be at least 1:0.2, preferably 1:0.5. It has now been found that SSL provides even better emulsifier results.

In this first step, the aqueous homogeneous mixture of the plant sterol and the emulsifier are homogeneously mixed to provide a micellar mix. The preferred mixing form is a high shear mixing. By way of example, vortexing, sonicating,

passing through a small orifice such as a French press or other mixing means may be employed. The most preferred mixing is sonication. This disperses the material and enhances the formation of a micellar mix that contains vesicles, the majority of which contain some plant sterol and some emulsifier.

Generally, with respect to sonication, any method that is commonly used for preparation of emulsions can be used to prepare homogeneous mixtures of the plant sterol and the emulsifier, either alone or in combination. For example, Waring blenders, or other high shear mixers can provide acceptable results. Microfluidizers can be used. In this latter procedure, the plant sterol and the emulsifier are forced through ceramic capillaries under high pressure. Where the preferred sonication technique is used, a time within the range of 1.5 minutes to about 4 minutes for sonication is sufficient. On small scale experiments, sonication is typically performed in about 1.5 minutes.

The drying process is not critical, so long as it does not destroy the vesicular complex formed between the plant sterol and the emulsifier. Generally, nondrastic drying procedures are preferred such as vacuum drying, freeze drying or low-temperature ambient air drying. Where heat is employed, the temperature at atmospheric conditions should not exceed 0°C.

As earlier explained, the dosage of the dry powder may be within the range of 10 to 1000 mg per day, and a preferred dose being 25 to 300 mg per day. The most preferred doses to achieve significant cholesterol absorption reduction levels are achieved at a dose range of from 100 mg to 300 mg one to four times daily.

The following examples are offered to further illustrate, but not limit the process of the present invention.

In these first five examples sitostanol is used as an example of a phytosterol and lecithin of a phospholipid as

per my parent application. In Example 6 SSL is used and the improvement can be seen.

Phytosterols, as used here, mean sterols such as sitostanol, sitosterol, campesterol, stigmasterol, saponins, lignans, aromatic and isoprenoid natural products, and their derivatives and reduction products. Phospholipids, as used here, means glycerophospholipids and sphingolipids, as well as their derivatives, such as lysophospholipids. Sodium stearoyl-2-lactylate (SSL) means this compound or its chemical equivalent reaction products of an alkali or alkaline earth metals and fatty acids and lactic acid.

EXAMPLE 1

Sitostanol, tracer amount of [³H]sitostanol, and other compounds that are found in the gut or that are commonly used as food additives were mixed together in chloroform solution at a fixed mole ratio. An aliquot, containing 1.2 μ mol of sitostanol, was transferred to an evacuation tube and the solvent was removed under reduced pressure (<50 mtorr). The experiment was initiated by adding 0.5 mL of artificial bile (8 mM sodium taurocholate containing 5 mM soy lecithin and 0.15 mM NaCl, pH 7.4) followed by rotation at 8 rev/min for 30 min at 37°C. The tube was then centrifuged for 1 minute at 17,000 xg to precipitate any solid material, the supernatant was removed and added to scintillation fluid for measurement of radioactivity, and the percent of radioactivity in the artificial bile supernatant was calculated. Table I below summarizes the solubility of sitostanol mixtures in the presence of artificial bile salt.

TABLE 1

Composition of Sterol Mixture Dried from Chloroform	Soluble Sterol, %
Sitostanol Alone	2.3
Sitostanol + Tween-20 1:1 by weight	7.8
Sitostanol + Taurocholate 1:1 by weight	57.7
Sitostanol + Monolein + Diolein 1:1:1 by weight	14.5
Sitostanol + Lecithin 1:1 by weight	38.2
Sitostanol + Lysolecithin 1:1 by weight	8.0
Sitostanol + Lecithin + Lysolecithin 1:1:0.5 by weight	97.9

As shown in line 1 of Table 1, sitostanol alone is poorly soluble in artificial bile salt (2.3%), and the addition of Tween-20, a polysorbate emulsifier used in foods, increases the solubility slightly to 7.8% (line 2). Sitostanol solubility can be enhanced 25-fold, from 2.3% to 57.7%, if it is dried in the presence of an ionic detergent, such as the bile salt sodium taurocholate (line 3). Since bile salt is a component of the digestive process, other compounds that are found in the gastrointestinal system were also tested. Monoolein and diolein are the products of dietary fat digestion, but as shown in line 4, they only produced a modest enhancement of solubility, 2.3% to 14.5%. Bile contains lecithin, and this phospholipid increased sitostanol solubility from 2.3% to 38.2% (line 5). However, the reaction product of phospholipase A₂ hydrolysis of lecithin, lysolecithin, produced a slight increase in sitostanol solubility, 2.3% to 8.0% (line 6). Surprisingly, when lecithin and lysolecithin were mixed together with sitostanol, the resulting solid mixture produced almost complete solubility of the sterol, 97.9% (line 7). Taken together, these data indicate that solid sitostanol does not readily dissolve in artificial bile, but that it can be made soluble to a varying degree by including other compounds in solid mixture. Moreover, a compound (lysolecithin) that by itself has little effect on sitostanol solubility can have a marked outcome when it is used in combination with other agents (lecithin).

EXAMPLE 2

Sitostanol, tracer amount of [³H]sitostanol and lecithin, were mixed together in chloroform. Two aliquots containing 1.2 μ Mol of sitostanol were removed and the chloroform solvent was removed under vacuum as described in Example 1. One aliquot was used without further preparation and to the other 500 μ l water was added and the sample mixture was sonicated for 5 minutes on 40% power with a Fisher Sonic Dismembrator Model 300 equipped with a microtip.

The sample was then frozen with dry ice acetone and lyophilized to remove water. It is essential to maintain the temperature of the sample below freezing in order to prevent precipitation of sitostanol from the mixture. The solubility of each of these samples in artificial bile was then determined as described in Example 1, and the results are shown in the Table below.

TABLE 2

Sample Drying Method	Soluble Sterol, %
Sitostanol dried from chloroform	2.3
Sitostanol/Lecithin(1:1 mole ratio) dried from chloroform	38.2
Sitostanol/Lecithin(1:1 mole ratio) sonicated in water, lyophilized	89.7

The data show the importance of lecithin in solubilizing sitostanol. However, the method of drying the sitostanol/lecithin mixture also affects the subsequent dissolution of the sterol. When the mixture is dried from chloroform, 38.2% of the sterol is solubilized by artificial bile. In contrast, when the mixture is sonicated and then lyophilized, solubilization increases to 89.7%. This shows that dispersing sitostanol/lecithin in aqueous medium followed by removal of water is a preferred method for preparing sitostanol/lecithin mixtures.

EXAMPLE 3

The effectiveness of variable amounts of lecithin to solubilize sitostanol was studied as in Example #1, except that after rotation at 37° for 30 min residual sedimenting sitostanol was re-extracted twice by vortexing with 0.5 ml additional artificial bile and recentrifuging. The following results were obtained:

TABLE 3

Sitostanol:Lecithin Mole Ratio	Soluble Sterol, %
1:1	53.1
1:2	67.9
1:10	67.6

These data show that even with repeated extraction and addition of a tenfold excess of lecithin, a significant amount of sitostanol (32%) remained insoluble. When [³H]phosphatidylcholine was added as a tracer instead of [³H]sitostanol, the amount of lecithin solubilized was 93.3%. This indicates that lecithin was nearly quantitatively extracted from the dried sitostanol/lecithin complex, whereas a limiting amount of sitostanol remained. Thus, methods to solubilize sitostanol in artificial bile must take into consideration the existence of residual insoluble sitostanol. Drying sitostanol/lecithin mixtures from a more polar solvent such as ethanol or a less polar solvent such as hexane gave similar results.

EXAMPLE 4

The effect of sonicated sitostanol/lecithin vesicles on human cholesterol absorption was compared to that of solid sitostanol dosed in the presence of sonicated lecithin. Sitostanol was dehydrated by twice dissolving in chloroform and evaporating, and was then ground to a powder in a mortar and pestle. To prepare the sitostanol/lecithin vesicles in a 1:3 mole ratio, 2.00 gm of sterol was added to 11.3 gm of purified soy lecithin in a 150 mL glass beaker. Chloroform was added with stirring to solubilize both components, and the solvent was then removed by incubating in a sand bath at 65°C. Soy lecithin (11.3 g) without sitostanol was prepared in the same manner. When all the solvent was removed, the beakers were placed in a lyophilization jar, and the residual chloroform was removed under vacuum for at least 24 hr. The solid in each beaker was then broken up with a spatula, 120

6651200-654205260

mL of deionized water was added, and the suspension was stirred vigorously for one hour. Vesicles were prepared by sonicating the contents of each beaker with a Branson Sonifier (setting 7) equipped with a small tip. During sonication, the beaker was immersed in a room temperature water bath. Vesicles containing lecithin alone were formed in 15-30 min, but those containing both sterol and lecithin required 30-45 min. The samples were then centrifuged at 10,000 xg for 10 min and passed through a 5 μ filter. The mean diameter of the vesicles determined on a Zetasizer that had been calibrated with a 250 nm standard was 204.7 nm for lecithin vesicles and 247.2 nm for the sitostanol/lecithin vesicles. The concentration of sitostanol was measured enzymatically. After preparation and characterization, the vesicles were stored overnight in a refrigerator at 4°C. The next day samples were diluted to 60 ml with water and 500 mg lemon flavored Crystal Light (Kraft Foods, Inc.) was added. Three U.S.P. stomach capsules were filled with a total of 1 g sitostanol powder or 1 g glucose placebo for each subject.

Six normal subjects underwent three cholesterol absorption tests in random order separated by 2 weeks. For each test a National Cholesterol Education Program Step 1 diet was consumed for 8 days beginning on day 1 of the study. On day 4, a standardized test breakfast was consumed consisting of 240 mL orange juice, 240 mL whole milk, 21 gm corn flakes and a 60 gm bagel saturated with 40 mg [26,26,26,27,27,27-²H₆] cholesterol tracer dissolved in 2.5 mL corn oil. Each subject also consumed a drink containing either sitostanol/lecithin vesicles or lecithin vesicles and three capsules containing either sitostanol powder or glucose placebo. The concentration of deuterated cholesterol tracer in plasma cholesterol on days 7 and 8 was measured by negative ion methane chemical ionization gas chromatography/mass spectrometry. Reduction in cholesterol absorption was determined by dividing the mean deuterated cholesterol concentration on days 7 and 8 by that observed during the test that contained only lecithin vesicles and

glucose capsules and expressing it as a percent. The following results were obtained:

TABLE 4

Treatment Given	Reduction in Cholesterol Absorption
1000 mg sitostanol powder	11.3±7.4%(p=0.2)
700 mg sitostanol/lecithin vesicles	36.7±4.2%(p=0.003)

These results show that, compared to placebo, 1000 mg sitostanol powder did not reduce cholesterol absorption significantly. This is consistent with previous reports showing that only multi-gram sitostanol doses reduce cholesterol absorption. However, 700 mg sitostanol/lecithin vesicles reduced cholesterol absorption by 37%, showing that properly formulated sitostanol is active and bioavailable.

EXAMPLE 5

To demonstrate that sitostanol/lecithin reduces cholesterol absorption in a pharmacological dose-response fashion, it was given in reduced amount to 5 of the 6 subjects of Example 4 during four additional cholesterol absorption tests. A dose of 300 mg sitostanol in sitostanol/lecithin vesicles was compared to lecithin placebo, and a dose of 150 mg sitostanol in sitostanol/lecithin vesicles was compared to another lecithin placebo. No capsules of solid sitostanol or placebo were given. The following results were obtained:

TABLE 5

Treatment Given	Reduction in Cholesterol Absorption
300 mg Sitostanol/Lecithin Vesicles	34.4±5.8%(p=0.01)
95 mg sitostanol/Lecithin Vesicles	5.6±7.2%(not significant)

Cholesterol absorption was reduced nearly as much by the 300 mg dose as the 700 mg dose, indicating that this dose is saturating. This is consistent with previous work showing that phytosterols do not completely block cholesterol absorption. There is no significant effect on cholesterol absorption at a dose of 95 mg.

EXAMPLE 6
The use of SSL (Sodium Stearoyl lactylate)

Since the parent patent application was filed in May, 1998, I have studied several emulsifiers used commonly in the food industry. The most useful for solubilizing sitostanol is SSL. SSL is potentially important because it is used in many foods including baked goods and has very acceptable taste and texture qualities when used with each food product.

In the following experiments collectively referred to as Example 6, sitostanol containing trace amount of ³H-sitostanol was dried from chloroform with a solubilizing agent, sonicated in water at a concentration of 2.4 mM sitostanol, frozen and lyophilized. Solubility in artificial bile was determined by adding 8 mM sodium taurocholate 5 mM PC 0.15 M NaCl 15 mM sodium phosphate pH 7.4 (artificial bile), rotating for 30 min. at 37° and centrifuging at 17,000 x g for 1 min. The pellet was washed once and the combined supers and pellet were counted separately. The results of triplicate experiments are shown below.

TABLE 6

condition	Mole Ratio	Percent Soluble
1. Sitostanol + SSL	1:0.90	95.0 ± 0.1
2. Sitostanol + SSL	1:0.68	94.5 ± 0.1
3. Sitostanol + SSL	1:0.45	92.8 ± 0.6
4. Sitostanol + lecithin	1:1	89.1 ± 1.2
5. Sitostanol + Precept 8160 central Soya PC/LPC ^a	1:1.2	95.7 ± 0.08
6. Ethoxylated MG ^b	1:1 by wt	82.5 ± 4.0
7. Sitostanol + Tween® 20 ^c	1:1 by wt	51.3 ± 2.8
8. Sitostanol + GMS ^d	1:1	Reaggregated after sonication
9. Sitostanol + MO ^e	1:1	46.4 ± 1.2

^a PC/LPC=lecithin/lyssolecithin

^b MG=monoglyceride

^c Tween® is registered trademark for polyoxyethylene-sorbitan monolaurate

^d GMS=glyceryl monostearate

^e MO=1-monoolein

In Table 6, trials 4-5 represent preferred compositions of my parent application. It can be seen that smaller amounts of SSL can be used to achieve comparable solubilities with an emulsifier of preferred taste. Thus, these experiments show that SSL is as effective as lecithin and lyssolecithin in solubilizing sitostanol. Monoglycerides, ethoxylated monoglyceride, and polysorbate 20 are less effective.

It can be seen from the above examples that the composition prepared in accordance with the process of this invention is bioavailable *in vitro* in bile, will significantly reduce cholesterol absorption, and that in general all of the objectives of the invention are achieved.

It should be understood that certain modifications should be and will be apparent to those of ordinary skill in the art, and that such modifications to the precise procedures in compositions set forth herein are intended to

come within the spirit and scope of the invention either literally or by doctrine of equivalents. In this light, the following claims are asserted.

09250769-1021599

What is claimed is:

1.

A composition for reducing cholesterol adsorption, comprising:

an aqueous homogeneous micellar mix of a plant sterol and an alkaline or alkaline earth metal salt of reaction product of lactic acid and a fatty acid as an emulsifier which has been dried to a finely divided water soluble powder;

the mole ratio of said plant sterol to emulsifier being within the range of 1:0.1 to 1:10.

2.

The composition of claim 1 wherein the mole ratio of plant sterol to emulsifier is at least 1:2.

3.

The composition of claim 1 wherein the mole ratio of plant sterol to emulsifier is within the range of 1:0.9 to 1:0.5.

4.

The composition of claim 1 wherein the micellar mix is a mix of vesicles, the majority of which contain some plant sterol and some emulsifier.

5.

The composition of claim 1 wherein the plant sterol is sitostanol.

6.

The composition of claim 1 wherein the metal is an alkali metal.

7.

The composition of claim 1 wherein the metal is sodium.

8.

The composition of claim 1 wherein the fatty acid is a carboxylic acid derived from an animal or vegetable fat or oil.

9.

The composition of claim 1 wherein the fatty acid is a C₄ to C₂₂ fatty acid.

10.

The composition of claim 1 wherein the fatty acid is selected from the group consisting of lauric, palmitic and stearic acid.

11.

The composition of claim 10 wherein the emulsifier is sodium stearoyl-2-lactylate.

12.

A method for reducing cholesterol absorption from food products, comprising the steps of:

adding finely divided water soluble powder formed from an aqueous homogeneous micellar mix of a plant sterol and alkali metal salt of reaction product of a lactic acid and a fatty acid as an emulsifier which has been dried to a food product;
the mole ratio of said plant sterol to said emulsifier of said powder being within the range of 1:0.1 to 1:10; the amount added to said food product being sufficient to provide a dose of from about 100 mg to about 1000 mg of sitostanol.

13.

The method of claim 12 wherein the dose is from 100 mg to 300 mg, provided at least one to four times daily.

14.

A food composition comprising:
a food including cholesterol and a food additive wherein the food additive is a water soluble homogeneous micellar mix of sitostanol and sodium stearoyl 2-lactylate emulsifier with a mole ratio of sitostanol to emulsifier being within the range of 1:0.1 to 1:10 in the added mix.

15.

The composition of claim 14 wherein the food product is solid food product.

16.

The composition of claim 14 wherein the food product is a beverage.

665120 65705260

ABSTRACT OF THE DISCLOSURE

Compositions useful to reduce cholesterol absorption. The compositions may be dosed in capsule or tablet form, or by adding either in liquid or dry powder form to foods and beverages. The compositions are an aqueous based homogeneous micellar mix which is dried to provide a mixture of finely-divided plant sterol, preferably sitostanol and lecithin. The mole ratio of the plant sterol, preferably sitostanol to lecithin, should be within the range of 1:0.1 to 1:10, preferably at least 1:2.

09250769.021599

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
COMBINED DECLARATION AND POWER OF ATTORNEY
FOR SOLE INVENTOR (C-I-P APPLICATION)

As the below named inventor I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor, of the subject matter which is claimed and for which a patent is sought on the invention entitled as follows: SITOSTANOL FORMULATION WITH EMULSIFIER TO REDUCE CHOLESTEROL ABSORPTION AND METHOD FOR PREPARING AND USE OF SAME, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code Of Federal Regulations, Section 1.56. I further declare that no application for patent or inventor's certificate on this invention has been filed by me, my legal representative or assigns in any country foreign to the United States of America except as identified below:

NONE.

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112.

I acknowledge the duty to disclose information that is material to the examination of this application, namely, information where there is substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

6657201 69409360

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS
DESIGNATING THE U.S. FOR BENEFIT
UNDER 35 U.S.C. § 120:

U.S. APPLICATIONS

Status (Check One)

Appln. No.	Filing Date		Patented	Pending	Abandoned
09/084,561	May 26, 1998			X	

PCT APPLNS. DESIGNATING THE U.S.

PCT Appln. No.	PCT Filing Date	U.S. Serial No. Assigned (if any)	Patented	Pending	Abandoned

And I hereby appoint ZARLEY, McKEE, THOMTE, VOORHEES & SEASE, comprising Donald H. Zarley, Registration No. 18,543; Bruce W. McKee, Registration No. 19,651; Dennis L. Thomte, Registration No. 22,497; Michael G. Voorhees, Registration No. 25,715; Edmund J. Sease, Registration No. 24,741; Mark D. Hansing, Registration No. 30,643; Kirk M. Hartung, Registration No. 31,021; Daniel J. Cosgrove, Reg. No. 36,770; Michael R. Crabb, Registration No. 37,298; Heidi Sease Nebel, Registration No. 37,719; Wendy K. Marsh, Registration No. 39,705; Jeffrey D. Harty, Registration No. 40,639; James A. Napier, Registration No. 42,025; and Mark Ziegelbein, Registration No. 43,307; 801 Grand Avenue, Suite 3200, Des Moines, Iowa 50309, Telephone 515-288-3667, as my attorneys to prosecute this application and to transact all business in the Patent Office connected therewith.

I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE

Inventor's signature:

Richard E. Ostlund Jr.
RICHARD E. OSTLUND, JR.

Date: 2-09-99

Full name of sole inventor: RICHARD E. OSTLUND, JR.

Residence: St. Louis, Missouri

Post Office Address: Washington University School of
Medicine
Division of Endocrinology
Diabetes and Metabolism
Campus Box 8127
660 South Euclid Avenue
St. Louis, Missouri 63110

Country of Citizenship: United States of America

This declaration ends with this page.